In the Specification

Please replace the first section title on page 1 with the following:

Related ~~Application~~ Applications

Please replace the second and third section titles on page 1 and paragraphs [0002] – [0008] as follows:

Technical Field ~~of the Invention~~

[0002] This ~~invention~~ disclosure relates to molecules binding to specific targets comprising Glu-Pro (EP) repeated motifs such, for example, the lymphocyte activation gene-3 (lag-3)-associated protein hereafter named LAP. The ~~invention~~ disclosure also relates to therapeutic compositions containing the molecules, antibodies directed against the molecules, to therapeutical compositions containing them. Also, the ~~invention~~ disclosure relates to methods for screening drugs useful for the treatment of immune disorders.

Summary ~~of the Invention~~

[0003] ~~This invention relates to~~ I provide a molecule binding to a target including an EP motif having the following sequence: $(X\text{-}(EP)_n\text{-}Y\text{-}(EP)_m\text{-}Z)_p$ wherein X, Y and Z may be identical or different and include a sequence of 0 to 10 amino acids, identical or different, n and m are integers between 0 to 20, ~~preferably~~ preferably between 3 to 10, with at least one of n or m being different from 0, and p is an integer between 1 and 10.

[0004] ~~This invention~~ I also ~~relates to~~ provide an expression vector including a nucleic acid molecule.

[0005] ~~This invention~~ I further ~~relates to~~ provide a method of treating immune-related pathologies including administering a therapeutically effective amount of a molecule to a patient in need thereof.

[0006] ~~This invention~~ I still further ~~relates to~~ provide a method for screening drugs including contacting a candidate drug with a molecule in the presence of a target EP motif and measuring resulting binding of the molecule to the target.

[0007] ~~This invention~~ I yet further ~~relates to~~ provide antibodies directed to a specific epitope of a polypeptide selected from the group consisting of polypeptides or peptides identified by SEQ

ID No:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6 and SEQ ID NO:9.

[0008] ~~This invention~~ I also further ~~relates to~~ provide a monoclonal antibody or a monoclonal antibody derivative that specifically binds a peptide selected from the group consisting of polypeptides or peptides identified by SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6 and SEQ ID NO:9, the monoclonal antibody derivative being selected from the group consisting of a monoclonal antibody conjugated to a cytotoxic agent or a radioisotope, and Fab, Fab' or $F(ab')_2$ fragments of the monoclonal antibody conjugated to a cytotoxic agent or radioisotope.

Please replace paragraph [0009] as follows:

Brief Description of the Drawings

[0009] The ~~invention~~ disclosure will be described in connection with experimental results and the Figures below wherein:

Fig. 1 represents the *in vitro* interaction of human LAP with hLAG-3;

Fig. 1A shows that LAP binds specifically to the natural hLAG-3 (70 kDa) protein present in whole cell lysate of PHA-activated human PBMCs;

Fig. 1B shows that LAP binds specifically to a protein produced by *in vitro* translation of an hLAG-3 mRNA in a rabbit reticulocyte lysate;

Fig. 2 illustrates interactions tested in the two-hybrid system using co-transformation with two plasmids and mating of two yeast strains;

Fig. 2A shows three partial LAP proteins (D1, D2 and D3) lacking their C-terminal domain were cloned in frame with the GAL4 AD protein, using a partial 1104 bp LAP cDNA;

Fig. 2B shows that the EP-rich C-terminal region of the PDGF receptor (PDGFR) was fused with the LexA BD;

Fig. 2C shows interactions in the two-hybrid system; and

Fig. 3 represents Western blots autoradiograme obtained with the anti-LAP immune serum, revealing a specific band at 45 kDa. Western blots were performed using 10 µl total cell lysates of PBMC (lanes 2, 4, 6) or PHA blasts (lanes 1, 3, 5). The blots were incubated in rabbit preimmune serum (lanes 1, 2), rabbit polyclonal antibody against LAP (lanes 3, 4) or the latter

preincubated with 10-6 M LAP peptide (lanes 5, 6). The arrow indicates the LAP 45 kDa protein.

Please replace paragraphs [0017] – [0031] as follows:

[0017] Thus, ~~the invention relates to~~ I provide molecules binding to a target comprising an EP motif, in particular, to molecules binding to a target comprising an EP motif having the following sequence:

$$(X\text{-}(EP)_n\text{-}Y\text{-}(EP)_m\text{-}Z)_p$$

wherein X, Y and Z may be identical or different and comprise a sequence of 0 to 10 amino acids, identical or different, n and m are integers between 0 to 20, preferably between 3 to 10, with at least one of n or m being different from 0, and p is an integer between 1 and 10.

[0018] In a preferred ~~embodiment, the invention relates to~~ aspect, I provide a molecule which binds to an EP motif selected from the group comprising the following formula: EPEPEPEPEPEPEPEPEP (SEQ ID N° 3), EPEPEPQLEPEP (SEQ ID N° 4), EPQDEPPEPQLELQVEPEPELEQ (SEQ ID N° 5), or EPEPEPEPEPEP (SEQ ID N° 6).

[0019] In another preferred ~~embodiment, the invention relates to~~ aspect, I provide a molecule that binds to an amino acid sequence comprising at least 5 EP motifs over a 19 amino acid length segment.

[0020] The molecule ~~of the invention~~ is selected from a peptide, a polypeptide or a protein. Preferably, the molecule is a purified polypeptide consisting of or comprising the amino acids sequence identified by SEQ ID No.:1, an ~~homologous~~ homolog, a fragment or a derivative thereof. More preferably, the molecule is a purified polypeptide consisting of or comprising the carboxy-terminal amino acids sequence of LAP identified by SEQ ID No.:2, an ~~homologous~~ homolog, a fragment or a derivative thereof.

[0021] ~~For the purpose of the invention:~~

~~an~~ An "homologous polypeptide" relates to a polypeptide or a protein which can differ by one or a few amino acid residues when compared with the polypeptide ~~of the invention~~, as the polypeptides identified by SEQ ID No. :1 or SEQ ID No. :2, but that maintain substantially all of the biological functions of the polypeptide, namely, its capacity to bind ~~glu pro~~ Glu-Pro motifs;

a A "polypeptide fragment" relates to any amino acid sequence contained in the sequence of the polypeptide ~~of the invention~~, which maintains the binding capacity for at Glu-Pro motifs; and

a A "polypeptide derivative" relates to the entire or fragment polypeptides, labelled with chemical or biological entities to be easily detected. Chemical or biological entities may be enzymes, fluorescent labels, ~~coloured~~ colored particles and the like.

[0022] ~~The invention also relates to a~~ The nucleic acid molecule ~~consisting~~ consists of or ~~comprising~~ comprises a polynucleotide sequence coding a polypeptide ~~according to the invention~~ and, particularly, to a nucleic acid molecule coding for the polypeptide identified by SEQ ID No. [[:]]:1. Also, the ~~invention relates to a~~ nucleic acid molecule[[,]]~~consisting~~ consists of or ~~comprising~~ comprises the polynucleotide sequence identified by SEQ ID No.:8, a fragment or a derivative thereof.

[0023] The ~~invention relates also to an~~ expression vector ~~comprising~~ comprises a nucleic acid molecule ~~according to invention~~. ~~For the purpose of the invention, an~~ An "expression vector" refers to any replicable DNA construct used either to amplify or express DNA, which encodes one of the polypeptides ~~of the invention~~.

[0024] ~~The invention~~ I also ~~relates to~~ provide a host cell transformed with an expression vector ~~according to invention~~. Host cells may be prokaryotic or eukaryotic, including but not limited to bacteria, yeasts, insect cells, mammalian cells, including cell lines, which are commercially available.

[0025] ~~The invention is~~ I also ~~directed to~~ provide a process for manufacturing a purified polypeptide comprising:

a) transfection of a host cell with an expression vector to obtain expression of the polypeptide, and

b) isolation and purification of the polypeptide from the transfected host cell.

[0026] Purification of the polypeptide may be accomplished by standard methods for purification of a membrane or soluble proteins. ~~The invention also relates to~~ I further provide a pharmaceutical composition comprising as an active agent at least one molecule ~~according to the invention~~. The pharmaceutical compositions ~~of the invention~~ are useful for treating immune-related pathologies and, in particular, they are useful for modulating immune responses. In a preferred ~~embodiment~~ aspect, the pharmaceutical compositions are useful to enhance

development of CD4 or CD8 T-cell populations. In another preferred ~~embodiment~~ aspect, the pharmaceutical compositions ~~of the invention~~ are also useful to suppress the development of CD4 or CD8 T-cell populations.

[0027] The pharmaceutical composition ~~of the invention~~ comprises as an active agent a LAP agonist. ~~In another preferred embodiment, the~~ The pharmaceutical composition ~~of the invention comprises~~ may comprise as an active agent a LAP antagonist. A LAP agonist is any molecule that mimics the effect of LAP binding when it binds to the target EP motifs and a LAP antagonist is any molecule that inhibits the affect of LAP binding when it binds to the target EP motif.

[0028] ~~The invention~~ I also ~~includes the~~ use ~~of~~ a molecule ~~according to invention~~ to manufacture a pharmaceutical composition useful for treating immune-related pathologies or for modulating immune responses. ~~The invention relates to the~~ I thus manufacture ~~of~~ a pharmaceutical composition enhancing the development of CD4 or CD8 T-cell populations. ~~The invention further relates to the~~ and manufacture ~~of~~ a pharmaceutical composition for suppressing development of CD4 or CD8 T -cell populations.

[0029] In a preferred ~~embodiment~~ aspect, the molecule is a LAP agonist. In a another preferred ~~embodiment~~ aspect, the molecule is a LAP antagonist.

[0030] ~~The invention~~ I also ~~includes~~ include a method for screening drugs comprising the steps of:

contacting the drug candidate with a molecule ~~according to the invention~~ in the presence of a target EP motif, and

measuring the resulting binding of the molecule to the target.

Please replace paragraphs [0032] – [0037] as follows:

[0032] ~~The invention~~ I also ~~relates to~~ provide antibodies directed to a specific epitope of the polypeptide identified by SEQ ID NO:1. ~~In preferred embodiments, the~~ The antibodies ~~are~~ may be monoclonal antibodies or polyclonal antibodies or Fab, Fab', F(ab') or Fv fragments thereof.

[0033] ~~The invention~~ I also ~~comprises~~ provide a monoclonal or polyclonal antibody or monoclonal or polyclonal antibody fragments or derivatives that specifically binds a peptide of SEQ ID NO:1, the monoclonal or polyclonal antibody derivative being selected from the group consisting of a monoclonal or polyclonal antibody conjugated to a cytotoxic agent or a

radioisotope, and Fab, Fab' or $F(ab')_2$ fragments of the monoclonal or polyclonal antibody conjugated to a cytotoxic agent or radioisotope.

[0034] Antibody fragments are regions from the polyclonal or monoclonal antibodies sequences ~~recognising~~ recognizing at least one epitope present in the peptide of SEQ ID NO:1, which maintain the binding capacity for at least one of the epitopes. Antibody derivatives are entire or fragment antibodies labelled with chemical or biological entities to be easily detected. Chemical or biological entities may be enzymes, fluorescent labels, ~~coloured~~ colored particles and the like.

[0035] ~~The invention relates~~ I also ~~to~~ provide a hybridoma cell line producing a monoclonal antibody ~~according to the invention~~. ~~The invention is also directed to a~~ A therapeutic composition ~~comprising~~ comprises as active ingredient an antibody ~~according to the invention~~. ~~The invention also relates to~~ I use ~~of~~ the antibodies in a method for purifying, identifying or quantifying a polypeptide or its homologs.

[0036] ~~The invention relates to~~ I use ~~of~~ the antibodies to screen compounds active in intracellular signaling mediated by cell surface receptor. ~~The invention~~ I also ~~relates to~~ use ~~of~~ the antibodies to screen compounds active in T-cell activation or regulation of the expansion of activated T-cells. ~~The invention is~~ I also ~~directed to~~ use ~~of~~ the antibodies to screen compounds active in platelet activation.

[0037] ~~The present invention also relates to~~ I further use ~~of~~ the antibodies for manufacturing a therapeutic composition useful for treating immune-related pathologies. ~~The invention also relates to~~ use ~~of~~ the antibodies for manufacturing an immunomodulatory pharmaceutical composition.